INVESTIGATION AND APPLICATIONS OF MICELLAR MOBILE PHASES IN REVERSED PHASE LIQUID CHROMATOGRAPHY

BY

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
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To my parents, Chun-Huang Lin and Lee-Won Lin

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First of all, I wish to thank my beloved parents; even though I am thousands of miles away from them, my heart is always filled with their love and care. Life in graduate school, whether academic or personal, is full of challenge. Without their understanding and encouragement, I could not have made it through.

Secondly, I would like to thank all the members in Dr. Dorsey's group. Because of them, I forgot homesickness, I learned many things ranging from American culture to science, and I also experienced how warm friendship can be. I will remember them all.

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Major Department: Chemistry

For micellar solutions as the mobile phases in reversed phase chromatography, the efficiency, some zwitterionic micelle behavior, adsorption isotherms, mobile phase strength, analytes' partitioning behaviors, and optimization of selectivity are examined. Microscopically, the micellar solution is a heterogeneous solution. The micelle aggregates, which have great solubilization power for water insoluble or less soluble compounds, offer an additional factor to the liquid chromatographic system to be manipulated.

Micellar mobile phases show greater decrease in efficiency for large hydrophobic molecules than hydroorganic mobile phases do, due to the stronger association of large hydrophobic molecules with micelles. The adsorption isotherms for cationic surfactants show constant stationary phase coverage by the surfactant molecules at concentrations above the critical micelle concentration, and the fast re-equilibration time for gradient elution with micellar mobile phase

would be expected. Generally, micellar mobile phases are weaker mobile phases than commonly used hydroorganic mobile phases, and among the same type of surfactants, the longer carbon chain ones give greater mobile phase strength than the shorter chain length ones. With the zwitterionic surfactant studied here, it appears that one of the ionic characters is absent. Optimization of separations based on secondary chemical equilibria gives liquid chromatography a powerful tool to achieve good selectivity for otherwise difficult separations. For micellar mobile phases, it seems that the optimization scheme is a mixed success, but at least it provides a guideline to be followed.

CHAPTER I
INTRODUCTION

Background

In reversed phase chromatography, an aqueous solution which is the combination of water and some organic modifier is used as the mobile phase, and the stationary phase which generally contains hydrocarbon coating on microparticulate silica is of hydrophobic character. The most used reversed phase columns are made of hydrocarbon chain chemically bonded onto the silica support (1, 2).

Analytes can be separated by liquid chromatography if they have differences in the distribution constant (K) between the mobile phase and the stationary phase. The retention can be described as the capacity factor (k') which can be related to the distribution constant and the retention time by the following equations,

$$k' = \frac{t_r - t_0}{t_0} \tag{1-1}$$

and

$$k' = \phi K \tag{1-2}$$

where t_0 is the elution time of an unretained analyte, t_r is the retention time of the analyte, and ϕ is the phase ratio of the column (volume of stationary phase/volume of mobile phase). The capacity

factor can also be related to some thermodynamic properties through temperature by the following equation,

$$\ln k' = -\frac{\Delta H_0}{RT} + \frac{\Delta S_0}{R} + \ln\phi \qquad (1\text{-}3)$$

where ΔH_0 and ΔS_0 are the enthalpy and entropy change of the analyte from mobile phase to stationary phase and R is the gas law constant. According to equation 1-3, the temperature has an effect on k', which is a thermodynamic factor itself; therefore, a constant temperature throughout the separation should be maintained.

The efficiency of the column is a way to measure the quality of the column, which can be estimated by the theoretical plate number, N. Another measure of the efficiency is the height equivalency of theoretical plate H, which is inversely proportional to the theoretical plate number N. The higher the N and the smaller the H, the better the efficiency the column has and also the better the resolution, R_s, is for adjacent peaks. N, H, and R_s can be calculated by the following equations (3, 4):

$$N = \frac{41.7(t_r/W_{0.1})^2}{B/A + 1.25} \qquad (1\text{-}4)$$

$$H = \frac{L}{N} \qquad (1\text{-}5)$$

$$R_s = \frac{1}{4} N^{1/2} \frac{\alpha - 1}{\alpha} \frac{k'}{1 + k'} \qquad (1\text{-}6)$$

where $W_{0.1}$ is the width of peak at 10% peak height, B/A is the asymmetry factor of the peak at 10% peak height, L is the length of

the column, and α is the selectivity factor for two adjacent peaks, which is the ratio of their capacity factors (always greater than or equal to 1). Another useful way to estimate the efficiency of a column is to use reduced plate height, h, which is the ratio of H over dp (stationary phase particle diameter). For a good high performance liquid chromatographic column operating at reasonable flow rate (less than 2 ml/min, for instance), the h value which is dimensionless is between 2 and 5. This provides a way to compare the efficiency of columns with different sizes of stationary phase packing. For a better efficiency column in certain liquid chromatography systems for the same analytes, the chromatographic peaks will be more symmetric in peak shape or narrower in base of the peak, or both, and R_s is greater.

Micellar Mobile Phase in High Performance Liquid Chromatography

Surfactants are amphiphilic molecules which contain a hydrophobic portion and a hydrophilic portion in the same molecule. According to the character of hydrophilic head groups, surfactants can be classified into ionic surfactant, which can be further divided into cationic and anionic surfactant; nonionic surfactant; and zwitterionic surfactant. The hydrophobic portion of the surfactant generally is a linear or branched hydrocarbon with between eight and twenty carbons (5). Over a narrow range of concentration there is a sudden change in the physical properties of aqueous surfactant solution. This concentration is termed critical micelle concentration (CMC)(6). At this concentration, the surfactant molecules start to aggregate with

hydrophobic portions associated with one another inside the micelle and the hydrophilic portion facing the aqueous environment. Micelles are dynamic aggregates. They can break up and aggregate again very rapidly. It takes between milliseconds and seconds to replace a whole micelle by this process (5). Microscopically, micellar solutions are heterogeneous, and that is the property of micelle aggregates that gives the unique feature of the separation. One of the most important properties of micellar solutions is their ability to solubilize substances which are insoluble or only slightly soluble in water. Sometimes the increase of the solubility of the sparingly water-soluble compounds can be several orders of magnitude (7).

Because of the micellar solution's being used for the first time as the mobile phase in high performance liquid chromatography (8), the interest in this field is increasing. Low efficiency of micellar liquid chromatographic separations prevents people from using them as an alternative to the hydroorganic mobile phase. With addition of 3% 1-propanol, the rate of mass transfer increases due to the better wetting of the stationary phase; the obtained efficiency is generally as good as that with the hydroorganic mobile phase (9). Gradient elution is an effective way to reduce the limit of detection and the separation time. However, the long re-equilibrium time of the system prevents it from being widely used. For a micellar solution, there is a nearly constant amount of free surfactant in the solution. Since they are at constant concentration, these surfactant molecules can adsorb onto the hydrophobic stationary phase. The adsorption isotherms of a few surfactants show constant coverage on the

stationary phase by the surfactants (10, 11, 12), and the micellar gradient elution separation without the column re-equilibrium time also has been demonstrated (10). The partition coefficient of a solute between 1-octanol and water serves as an indicator for the prediction of pharmacological activity. With micellar liquid chromatography which is without any organic modifier, the chromatographic behavior of a series of monosubstituted benzenes has good correlation with the partition coefficients obtained from a standard system which is 1-octanol/water (13). As mentioned earlier, a micelle aggregate has great solubilization power. With this property, the direct body fluid injection into micellar liquid chromatography for therapeutic drug monitoring has been achieved (14, 15). Apparently micellar liquid chromatography is not only just for the laboratory curiosity, it also has practical applications.

CHAPTER II
EXPERIMENTAL

Liquid Chromatography System

The high performance liquid chromatographic systems used in these experiments were composed of a high pressure pump, an injector, a reversed phase chromatographic column, a detector, and a strip chart recorder. The high pressure pump was either Altex (Beckman Instruments, San Ramon, California) model 100A pump or a Spectra-Physics (San Jose, California) SP8700 extended range pump. The injector was either a Valco Instruments Co. (Houston, Texas) model C6U or a Rheodyne (Cotati, California) 7125 with 10 µl and 20 µl loops. The reversed phase chromatographic column was either a Phenomenex Ultrex 5 C-18 [2 mm internal diameter (i.d.) x 15 cm length, 5 µm particle diameter] or a Brownlee Spheri-5 C-8 or C-18 (4.6 mm i.d. x 10 cm length, 5 µm particle diameter) cartridge column, or an Altex Ultrasphere C-8 or C-18 (4.6 mm i.d. x 15 cm length, 5 µm particle diameter) column. During the experiment, the column was encapsulated in a glass water jacket which was maintained at constant temperature by a Fisher (Pittsburgh, Pennsylvania) model 73 immersion circulator and water bath. A 4.6 mm i.d. x 12 cm length precolumn was used in all of the experiments except for the adsorption isotherm measurements. It was connected between the pump and injector and was

dry hand-packed with 25-40 μm silica (Macherey-Nagel and Co., Duren, Germany). The detector was either a Kratos (Ramsey, New Jersey) Spectroflow 757 UV/VIS variable wavelength absorbance detector, a LDC/Milton Roy (Riviera Beach, Florida) ConductoMonitor III conductivity detector or a Perkin-Elmer (Norwalk, Connecticut) TriDet detector. The strip chart recorder was either a Fisher D-5000 strip chart recorder or a Perkin-Elmer R50 recorder.

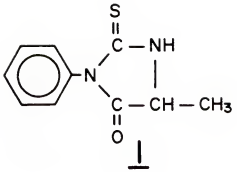
Reagents

The surfactants used in the experiments were polyoxyethylene laurylether (Brij-35) from Fluka (Ronkonkoma, New York) and Aldrich (Milwaukee, Wisconsin); sodium dodecyl sulfate (SDS) from Fluka and Calbiochem (Behring Diagnostics, La Jolla, California); tetradecyltrimethylammonium bromide (C14TAB) from Fluka and Chemical Dynamic Corporation (South Plainfield, New Jersey); hexadecyltrimethylammonium bromide (CTAB) from Fluka and Chemical Dynamic Corporation; octadecyltrimethylammonium bromide (C18TAB) from Fluka; and a zwitterionic surfactant, N-hexadecyl N,N-dimethyl-3-ammonio-1-propane sulfonate (Z3-16), from Calbiochem. The surfactants were used as received. Methanol and acetonitrile (both HPLC grade), citric acid monohydrate, and 1-propanol were from Fisher Scientific (Fairlawn, New Jersey). Water used in the mobile phases was from a Barnstead Nanopure system (Boston, Massachusetts), irradiated for at least 48 h by ultraviolet light in a Photronix model 816 HPLC reservoir (Medway, Massachusetts) to remove trace organics. The surfactant solutions were all filtered through 0.45 μm Nylon-66 membrane filters (Rainin

Instruments, Woburn, Massachusetts, and Alltech Associates, Inc., Deerfield, Illinois) prior to use in the LC system. The solutes used were certified grade aniline and acetophenone (Fisher Scientific); reagent grade phenol, benzene, and sodium benzoate (USP powder) (all Mallinckrodt, St. Louis, Missouri); reagent grade naphthalene; nitrobenzene (all Eastman); anthracene (Matheson Coleman and Bell); pyrene (Chemical Service, West Chester, Pennsylvania); and twenty different phenylthiohydantoin (PTH) amino acids (Sigma, St. Louis, Missouri) (which are listed in Table 2-1 and illustrated in Fig 2-1). Solutes were dissolved in pure methanol or acetonitrile and then diluted to a suitable injection concentration with a solution of the same composition as the mobile phase for efficiency, adsorption isotherm measurements, zwitterionic surfactant experiment, and PTH-amino acids optimization, only HPLC grade methanol was used to prepare the analytes.

Table 2-1 List of phenylthiohydantoin amino acids (PTH-amino acids)

Reference Number	Compound	Molecular Weight (g/mole)
1	PTH-Alanine	206.3
2	PTH-Arginine hydrochloride	327.8
3	PTH-Asparagine	249.3
4	PTH-Aspartic acid	250.3
5	PTH-S-Carboxymethylcysteine	296.4
6	PTH-Glutamic acid	264.3
7	PTH-Glutamine	263.3
8	PTH-Glycine	192.2
9	PTH-Histidine	272.3
10	PTH-Isoleucine	248.4
11	PTH-Leucine	248.4
12	N-α-PTH-(ε-Phenylthiocarbamyl)-lysine	398.5
13	PTH-Methionine	266.4
14	PTH-Phenylalanine	282.4
15	PTH-DL-Proline	232.3
16	PTH-Serine	222.3
17	PTH-Threonine	236.3
18	PTH-Tryptophan	321.4
19	PTH-Tyrosine	298.4
20	PTH-Valine	234.3



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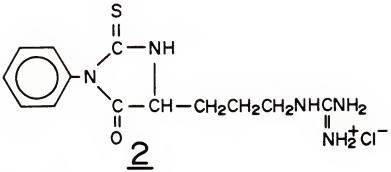

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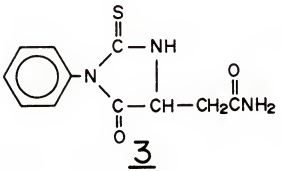

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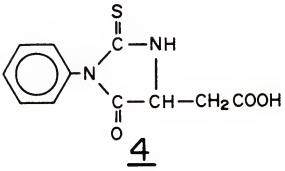

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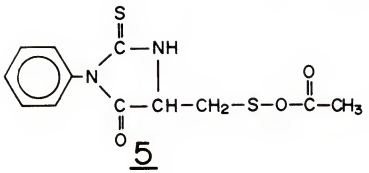
Fig. 2-1 Structure of twenty PTH-amino acids



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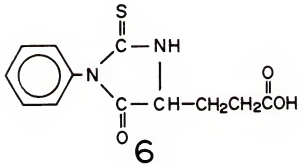

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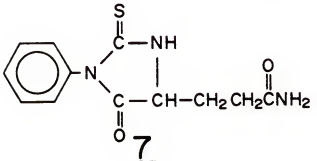

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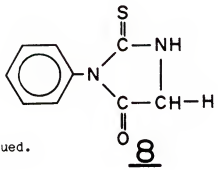

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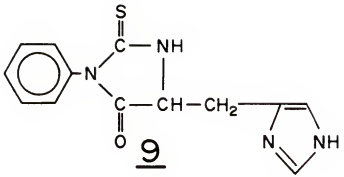
Fig. 2-1--continued.



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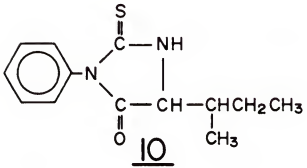

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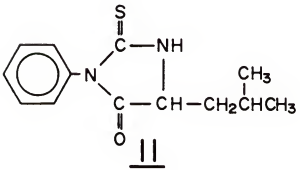

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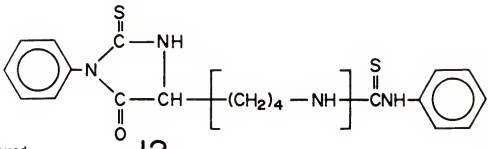

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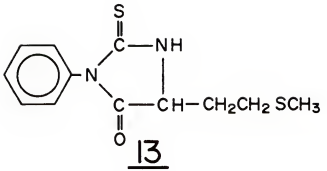

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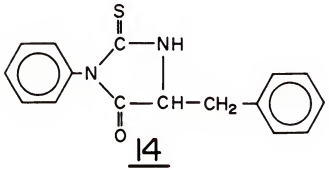

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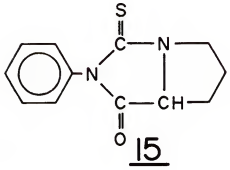

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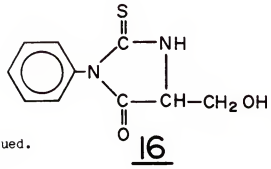

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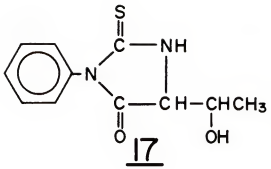

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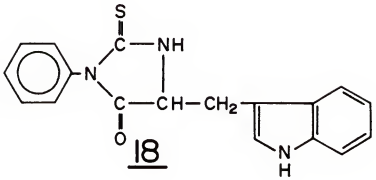

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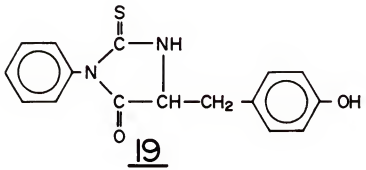

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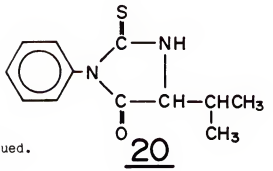

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Fig. 2-1--continued.

CHAPTER III
MICELLAR LIQUID CHROMATOGRAPHY

Comparison of Hydroorganic and Micellar Mobile Phase

By far, reversed phase liquid chromatography (RPLC) is the most widely used liquid chromatographic mode. By using hydroorganic mobile phases, RPLC offers good chromatographic efficiency and versatility.

In liquid chromatographic systems, the analytes exchange between the mobile phase and the stationary phase; hence, separation is achieved by the differences in the partition coefficients of the analytes. Therefore, the retention of analytes is governed by analytes' thermodynamic properties. If only the difference in thermodynamic properties exists, the efficiency of each separated peak will be the same. If there is a difference in dynamic properties, the efficiency for the analytes under the same separation condition will be different. Will using hydroorganic mobile phase and micellar mobile phase make any difference in efficiency?

The micellar liquid chromatography, due to the larger resistance to mass transfer, has lower efficiency than liquid chromatography with hydroorganic mobile phase (16). But after the elevation of the column temperature (40°C), which increases the kinetic process, and the addition of 3% 1-propanol to the micellar mobile phase, which increases the mass transfer by wetting the stationary phase (C-18), the efficiency attained is at least equivalent to that of the

hydroorganic counterpart (9). This result was disputed by other researchers who claimed that the addition of organic modifier did not have the effect of increasing efficiency and the efficiency enhancement worked only for analytes having high exit rate constants, which are the rate constants for the processes of analytes dissociating from micelle aggregates, such as aromatic compounds (17). Those experiments were done by using C-1 column with aromatic and polyaromatic compounds as analytes. For the addition of organic modifier, because the C-1 stationary phase, which is more easily accessed by mobile phase than C-18 stationary phase, is considerably less hydrophobic, no or much less stationary phase wetting problem will occur and no or very little improvement in efficiency on C-1 column on addition of organic modifier is expected. For the efficiency measurement, is the low efficiency typical only for micellar mobile phase with polyaromatic analytes? And how does the hydroorganic mobile phase affect the efficiency for aromatic and polyaromatic compounds? The following experiment was conducted in order to answer the above questions.

In this experiment, 30/70 and 70/30 (volume/volume, acetonitrile/water solutions), with 15 cm, C-8 column were the hydroorganic systems, and [CTAB] = 0.154 M in 3/97 (v/v, 1-propanol/water) buffered at pH = 3 by 0.01 M citric acid with a 10-cm C-18 column was the micellar system; all were at kept at 30°C by circulating the constant temperature water through the column water jacket. Several aromatic and polyaromatic solutes were used in this experiment.

The results are shown in Tables 3-1, 3-2, and 3-3. In the 70/30 case (Table 3-1), as the molecular size goes up, the efficiency goes

Table 3-1 Efficiency for hydroorganic mobile phase with high percentage of organic modifier

Flow rate (ml/min)	Benzene		Naphthalene		Anthracene		Pyrene	
	N	h	N	h	N	h	N	h
0.46	9150	3.3	9540	3.1	8610	3.5	8390	3.6
0.76	10650	2.8	10320	2.9	9250	3.2	8640	3.5
0.96	9930	3.0	10230	2.9	8990	3.3	7780	3.9
1.17	10250	2.9	10100	3.0	9330	3.2	7840	3.8
1.48	9880	3.0	9860	3.0	8860	3.4	7770	3.9
1.79	9670	3.1	9890	3.0	8890	3.4	7490	4.0
1.92	9600	3.1	9670	3.1	8580	3.5	7170	4.2

Mobile phase: 70/30 (acetonitrile/water), 30°C

Stationary phase: Altex, 5 µm, C8, 4.6 mm i.d. x 15 cm

Table 3-2 Efficiency for hydroorganic mobile phase with low percentage of organic modifier

Flow rate (ml/min)	Nitrobenzene		Benzene		Naphthalene	
	N	h	N	h	N	h
0.51	7650	3.9	5300	5.7	4420	6.9
0.80	8610	3.5	7800	3.8	5840	5.1
1.00	8750	3.4	7950	3.8	6320	4.7
1.29	9820	3.1	9170	3.3	6990	4.3
1.42	9070	3.3	9190	3.3	6660	4.5
1.74	8200	3.7	9490	3.2	7400	4.1
1.90	9180	3.3	8350	3.6	6990	4.3
2.40	9270	3.2	8590	3.5	7390	4.1
2.99	9390	3.2	9690	3.1	7510	4.0

Mobile phase: 30/70 (acetonitrile/water), 30°C

Stationary phase: Altex, 5 µm, C8, 4.6 mm i.d. x 15 cm

down. The same trend holds for the 30/70 case (Table 3-2) and CTAB micellar mobile phase (Table 3-3). The efficiency of some analytes was plotted as H vs flow rate (Figs. 3-1 to 3-6). It is clear that under the same conditions, the larger molecule has lower efficiency. For the hydroorganic mobile system, this phenomenon can be explained in terms of diffusion coefficient for the analytes. According to the van Deemter equation, for a well-packed modern analytical liquid chromatographic column, which has chemically bonded stationary phase on small porous particles, with reasonable mobile phase flow rate, the efficiency will increase as the analyte's diffusion coefficient increases. The diffusion coefficients are listed in Table 3-4 and were calculated from the Wilke-Chang equation (18),

$$Dm = \frac{7.4 \times 10^{-8} (\psi_2 M_2)^{0.5} T}{\eta V_1^{0.6}} \qquad (3\text{-}1)$$

where Dm is the diffusion coefficient (cm^2/s) of the analyte in the mobile phase, ψ_2 is the association factor which is 2.6 for water and 1 for acetonitrile (18) of the mobile phase, M_2 is the molecular weight of the mobile phase in g/mole, T is temperature in °K, η is the viscosity of the mobile phase in centipoise (cP), and V_1 is the molar volume of the analyte in ml. ψ_2 and M_2 are estimated from the molar fraction of the mobile phase components.

From Tables 3-1 to 3-4, it can be seen that in the three different mobile phase systems, the larger molecules have lower diffusion coefficients and therefore also have lower efficiency. Comparing the efficiency of analytes in micellar mobile phase and in

Table 3-3 Efficiency for micellar mobile phase

Flow rate (ml/min)	Benzene		Anthracene		Pyrene	
	N	h	N	h	N	h
2.0	3140	6.4	470	43	130	150
1.0	4040	5.0	800	25	260	77
0.75	4740	4.2	980	20	350	57
0.50	4620	4.3	1350	15	530	38

Mobile phase: [CTAB] = 0.154 M, in 3/97 (1-propanol/water), pH = 3, 0.01 M, citric acid buffer, 30°C

Stationary phase: Brownlee, 5 μm, C18, 4.6 mm i.d. x 10 cm

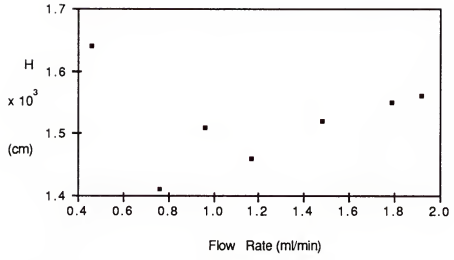


Fig. 3-1 H vs flow rate plot for benzene in 70/30 (acetonitrile/ water) mobile phase

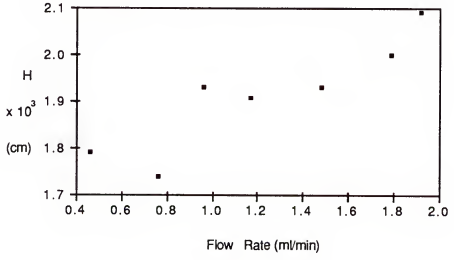


Fig. 3-2 H vs flow rate plot for pyrene in 70/30 (acetonitrile/ water) mobile phase

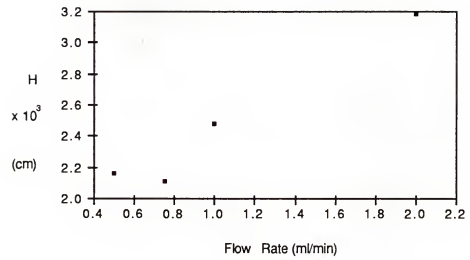


Fig. 3-3 H vs flow rate plot for benzene in [CTAB] = 0.154 M, pH = 3 mobile phase

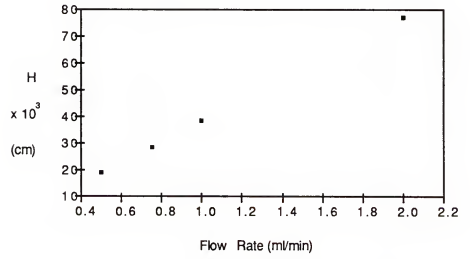


Fig. 3-4 H vs flow rate plot for pyrene in [CTAB] = 0.154 M, pH = 3 mobile phase

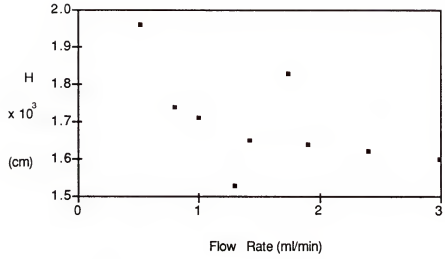


Fig. 3-5 H vs flow rate for nitrobenzene in 30/70 (acetonitrile/ water) mobile phase

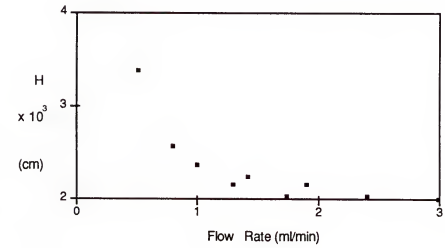


Fig. 3-6 H vs flow rate for naphthalene in 30/70 (acetonitrile/ water) mobile phase

Table 3-4 Calculated diffusion coefficient

Compound	Dm (cm^2/s)
70/30, CH_3CN/H_2O, 30°C Ψ_2 = 1.92, M_2: 27.76, η: 0.67 cP	
Benzene	$1.7x10^{-5}$
Naphthalene	$1.5x10^{-5}$
Anthracene	$1.3x10^{-5}$
Pyrene	$1.2x10^{-5}$
30/70, CH_3CN/H_2O, 30°C Ψ_2 = 2.41, M_2: 20.75, η: 0.95 cP	
Nitrobenzene	$1.0x10^{-5}$
Benzene	$1.1x10^{-5}$
Anthracene	$8.5x10^{-6}$

hydroorganic mobile phases, the efficiency of larger molecules, with respect to benzene, have the greater decrease of efficiency in micellar mobile phase than in hydroorganic mobile phases. Micellar solutions are more viscous than hydroorganic ones; therefore, a lower efficiency resulting from the higher mobile phase viscosity might be expected if all other factors are kept the same. But from the sharp drop of the efficiency for larger molecules, other existing influences besides viscosity should be very likely. Compounds, such as anthracene and pyrene which are more hydrophobic than benzene, have larger equilibrium constants between the micelle and bulk solvent (19) and tend to stay in the micellar aggregate longer. Additionally, the diffusion coefficient of CTAB micelles in aqueous solution is in the low 10^{-6} cm^2/s region (20), thus causing even greater decrease in efficiency for more hydrophobic analytes. The possibility of multiple encounters of micellar aggregates (17) also makes the mass transfer less likely, reducing the efficiency even further. Although the micellar solution used in this study is not at optimized conditions, these experiments show that the micellar mobile phase causes decreases of efficiency for larger hydrophobic molecules to be greater than the decreases caused by the hydroorganic mobile phase.

Zwitterionic Surfactant

Micellar liquid chromatography has been utilized to separate analytes ranging from ionic (21, 22) to neutral (16, 23). By using the ionic surfactant mobile phase which has the concentration below CMC, the oppositely charged analytes can be separated by the enhancement of the retention in reversed-phase ion-pair

chromatography. Since the ionic micellar solutions are able to separate the ionic and neutral analytes, there is the possibility that the zwitterionic micellar mobile phases are able to interact with ionic, zwitterionic, and neutral analytes to achieve the separation under one run of the experiment. Some zwitterionic surfactants have been used in zwitterion-pair chromatography to achieve the separation of nucleotides (24), and some aspects of the mechanism also have been discussed (25, 26). In this experiment, a zwitterionic surfactant, N-hexadecyl-N,N-dimethyl-3-ammonio-1-propan-sulfonate (Z3-16) which is pH insensitive (27), was used by dissolving appropriate amount into 3/97 (1-propanol/water). The column used was C-18, 5 μm, 2 mm i.d. x 15 cm, and was kept at 30°C by a constant temperature water bath. The test compounds used were benzene (a neutral compound), sodium benzoate (pKb = 9.80) (a basic compound), anilinium chloride (pKa = 4.60) (an acidic compound), and tyrosine (pKa = 2.20, 9.11, and 10.07) (a zwitterionic compound).

An attempt was made to determine the adsorption isotherm for the surfactant; however, this was not successful, because the CMC for the surfactant is only in the range of $3x10^{-5}$ M. The conductivity detector is not sensitive enough to have response at that small concentration. The next attempt was to estimate the retention for several analytes mentioned above. Those results were plotted as capacity factor (k') vs surfactant concentration in Figs. 3-7 to 3-12.

Those figures show Z3-16 surfactant acting exactly like the anionic surfactant. In the sodium ion (Fig. 3-7) case, the sodium ion shows a certain amount of retention, and the retention is attributed

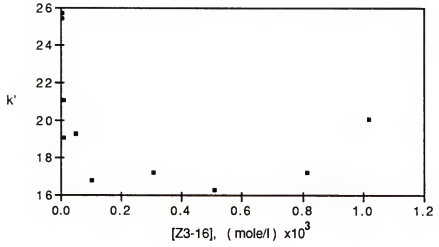


Fig. 3-7 k' vs [Z3-16] plot for sodium ion (conductivity)

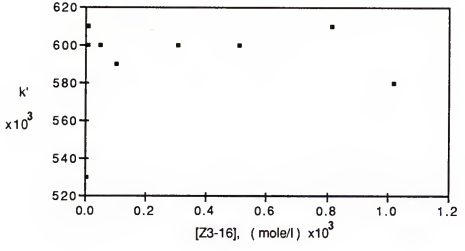


Fig. 3-8 k' vs [Z3-16] plot for chloride ion (conductivity)

to the surfactant modified stationary phase carrying the opposite charge, and as the micelle concentration increases, the retention decreases. The capacity factors of the chloride ion (Fig. 3-8) stay very close to zero, which means chloride ions do not interact with the stationary phase or just very little, and this implies the stationary phase is either hydrophobic, which is disproved by the sodium ion case, or negatively charged. The anionic character of the surfactant can also be demonstrated by the anilinium ion (Fig. 3-9) and the benzoate ion (Fig. 3-10), which show the much longer retention of the cationic character anilinium than the anionic character benzoate ion with each having a phenyl group and an ionic group in the molecule. For tyrosine (Fig. 3-11), since the pH value of the micellar solutions is in the vicinity of 6 (Table 3-5), tyrosine is zwitterionic under this pH, and it shows the capacity factor around 1 in the concentration range employed. For benzene (Fig. 3-12), it shows that as the micelle concentration increases the retention decreases very slightly. Also from some test tube trials, when the zwitterionic surfactant solution was mixed with either 0.1 M Brij-35 solution or 0.2 M SDS solution, solution was still visually clear, when it was mixed with 0.3 M C14TAB solution, the solution turned cloudy. This also shows that the Z3-16 surfactant is acting more like an anionic surfactant. From the observations above, it seems that the cationic character of the tetraalkyl ammonium group was overshadowed by the anionic sulfonate group, and also since the Z3-16 surfactant is pH insensitive but only anionic character appeared, the steric hindrance factor might play an important role in the ionic interactions.

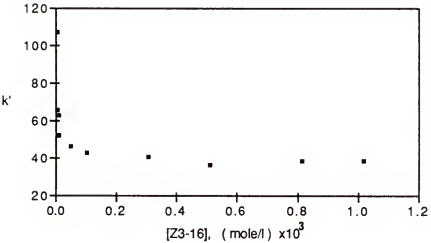


Fig. 3-9 k' vs [Z3-16] plot for anilinium ion (UV)

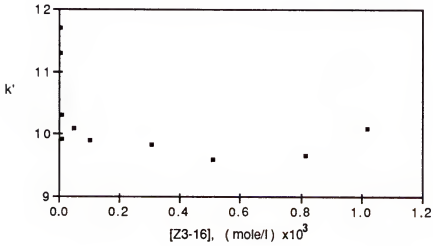


Fig. 3-10 k' vs [Z3-16] plot for benzoate ion (UV)

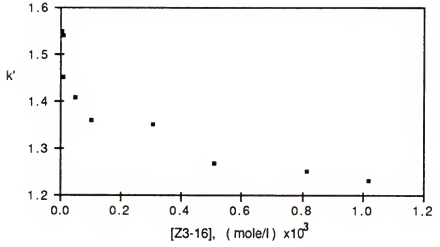


Fig. 3-11 k' vs [Z3-16] plot for tyrosine (UV)

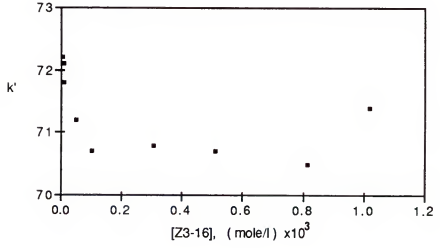


Fig. 3-12 k' vs [Z3-16] plot for benzene (UV)

Table 3-5 pH Values for Z3-16 surfactant solutions

[Z3-16], M	pH
5.09×10^{-7}	6.20
1.02×10^{-6}	5.89
1.27×10^{-6}	6.11
2.71×10^{-6}	6.31
4.07×10^{-6}	6.19
5.09×10^{-6}	6.03
6.78×10^{-6}	6.04
1.02×10^{-5}	5.64
5.09×10^{-5}	6.01
1.02×10^{-3}	6.00

Cationic Surfactants

The most commonly seen hydrophobic portion in the surfactant molecule is the alkyl chain. It imparts some special properties to the micelles. For the longer alkyl chain, the CMC is lower (28). Also for solubilization process in micellar solutions, the ability to solubilize the moderately hydrophobic reagents is greater with longer alkyl chain micelle, while the chain length has no effect on solubilization of copper ion (29). In reversed phase liquid chromatography, hydrophobic compounds will elute late during the separation compared to less hydrophobic ones; therefore, the separation time is determined by the latest eluted compound. If one can speed up the retention of more retained, also more hydrophobic, compounds with little or no alteration of the early eluted compounds, then the speed and also the quality of separation can be enhanced. Since the previous example (29) gave this promising clue for the micellar solution, further study of the applicability in micellar liquid chromatography was needed. Hexadecyltrimethyl ammonium bromide (CTAB) is one of the most widely used surfactants. In the following studies, C_{14}TAB, CTAB, and C_{18}TAB are used, and the possibility of better separation and the surfactant chain length effects can also be explored.

Adsorption Isotherms

Three cationic surfactants, C_{14}TAB, CTAB, and C_{18}TAB, were used in this study. Appropriate amounts of each surfactant were dissolved in a 3% 1-propanol and 97% water (V/V) mixture, and low concentration

solutions were made by suitable dilution. Chromatographic columns used were all octyl bonded with a 5 μm particle size and either 10 cm or 15 cm in length.

The adsorption isotherm measurements were done at 30°C by using the frontal analysis technique (30, 31). In order to detect ionic surfactants, the conductivity detector was used. A certain concentration of surfactant solution was introduced continuously through the HPLC system, until the mobile phase and stationary phase reached equilibrium and a "breakthrough" signal was obtained (Fig. 3-13). From the breakthrough signal, the amount of the surfactant adsorbed onto the stationary phase could be estimated by the following equation:

$$q(C_{a,n}) = q(C_{a,n-1}) + [(C_{a,n} - C_{a,n-1})(V_{b,n} - V_v)]/A_{sp} \qquad (3\text{-}2)$$

where $q(C_{a,n})$ and $q(C_{a,n-1})$ are the accumulated surface concentration of adsorbed surfactant (mole/m^2) after the nth or (n-1)th elutions, $C_{a,n}$ and $C_{a,n-1}$ are the concentrations (mole/l) of surfactant mobile phase at the nth and (n-1)th elutions, $V_{b,n}$ (ml) is the breakthrough volume at nth elution, V_v (ml/column) is the void volume of the column (which was determined by measuring the start of first perturbation of detector response after injecting water), and A_{sp} (m^2/column) is the total surface area of the stationary phase in the LC column used (Table 3-6). The adsorption isotherms were constructed by plotting $q(C_{a,n})$ vs $C_{a,n}$ (Figs. 3-14, 3-15, 3-16, and 3-17). The surface coverage of octyl ligand of the stationary phase, a, (μmole/m^2) was

Table 3-6 Specification of two types of octyl columns

Column	Surface Area, m^2/g	Weight of Stationary Phase, g	Carbon Loading, %	Surface Coverage of Octyl Ligand, $\mu mole/m^2$
Altex, Octyl Ultrasphere 4.6 mm i.d. 15 cm, 5 μm	200	2.25	6.5	2.98
Brownlee Spheri-5 4.6 mm i.d. 10 cm, 5 μm	320	1.75	8.0	2.35

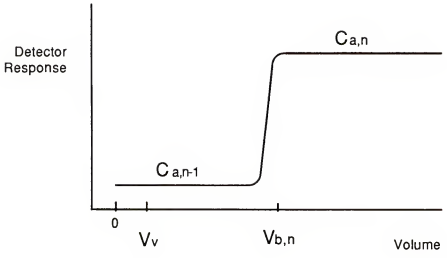


Fig. 3-13 Breakthrough method

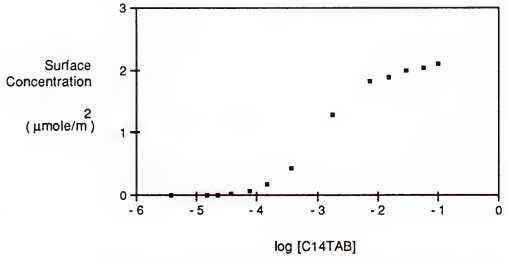


Fig. 3-14 Adsorption isotherm for C14TAB at 30°C

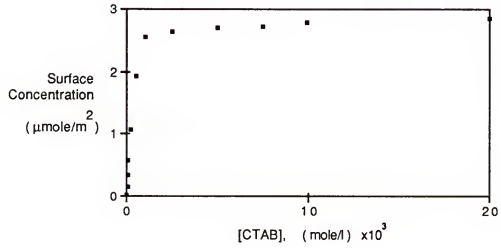


Fig. 3-15 Adsorption isotherm for CTAB at 30°C

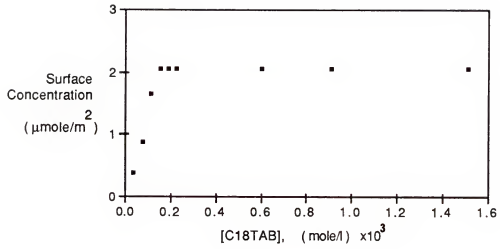


Fig. 3-16 Adsorption isotherm for C18TAB at 30°C

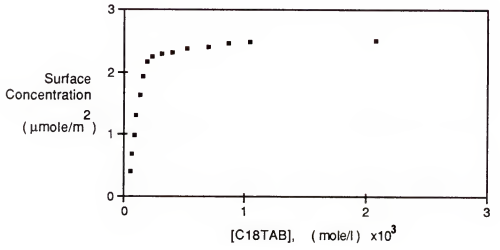


Fig. 3-17 Adsorption isotherm for C18TAB at 40°C with 10-cm column

calculated by the following equation (private communication with Dr. Karen Sentell):

$$a = \frac{(\%C)(10^6)}{(12.011)(n_c)(S)[100 - [(\%C)/(12.011)(n_c)](M - 36.5)]} \quad (3\text{-}3)$$

where n_c is the number of carbon atoms per bonded silyl ligand (ten in this case), M is the molecular weight of the silyl ligand, S (m^2/column) is the total surface area of the stationary phase (private communication with manufacturer), and %C is the percent carbon loading of the stationary phase (private communication with manufacturer).

From the adsorption isotherms (Figs. 3-14, 3-15, 3-16, and 3-17), the CMC values and surface coverage of the stationary phases were obtained (Table 3-7). Below the CMC, the surface coverage by the surfactant increased as the surfactant concentration of the mobile phase increased. This occurs, because below the CMC, increasing the surfactant concentration increases the amount of free surfactant molecules, which are responsible for surface coverage; therefore more surfactant molecules will be available for adsorption onto the stationary phase. Therefore, the adsorption isotherm shows a sharply rising edge in the beginning. At the CMC, the free surfactant molecules start to aggregate and form micelles. As more surfactant was added to the solution, the micelle concentration (which equals [surfactant] - CMC) increases, while the free surfactant concentration kept fairly constant. Since only free surfactant molecules are responsible for adsorption, the absorption isotherm reached a plateau above the CMC. Since the columns were equilibrated to constant adsorption coverage of cationic surfactants (C14TAB, CTAB, and C18TAB)

Table 3-7 Critical micelle concentration determined by adsorption isotherm

Surfactant	Critical Micelle Concentration (CMC)	Surface Coverage ($\mu mole/m^2$)	% Surface Coverage
C14TAB*	1.4×10^{-2} M (3.5×10^{-3} M)***	1.98	66
CTAB*	6.7×10^{-4} M (9.2×10^{-4} M)	2.65	89
C18TAB*	1.5×10^{-4} M (3.0×10^{-4} M)	2.06	69
C18TAB**	1.8×10^{-4} M (3.0×10^{-4} M)	2.25	96

* 15-cm column

** 10-cm column

*** Literature values from reference 32.

with micellar solutions, gradient elution chromatography using micellar mobile phases should require no column re-equilibrium after each gradient run (10). With longer carbon chain length surfactants, the hydrophobicity of the surfactant increases. In order to reduce the exposure of the hydrophobic portion of the surfactant molecule to the hydrophilic media, longer carbon chain length surfactants start forming micelles at lower concentrations than shorter carbon chain length surfactants having the same hydrophilic portion. As a general rule, for ionic surfactants in an aqueous medium, the CMC is halved by the addition of one methylene group to a straight-chain hydrophobic portion which is attached to a single terminal hydrophilic portion (27). Table 3-7 shows that for the cationic surfactants used in these experiments, the CMC decreases as the carbon chain length of the surfactant increases, which roughly conforms to the rule stated above. The CMC values obtained from the adsorption isotherms are different from literature values (Table 3-7); mainly the difference is attributable to the addition of 3% 1-propanol to these systems, which makes the aqueous solution more hydrophobic. From Figs. 3-14, 3-15, 3-16, and 3-17, it can be seen that the surfactant adsorption isotherms are very similar in shape (L-shaped) (33) to a Langmuir isotherm. The adsorption isotherm data were fitted to a linearized form of the equation for the Langmuir isotherm in order to see if the adsorption isotherms conformed to Langmuir behavior. The linearized form is

$$\frac{1}{C_s} = \frac{a}{(C_m)(C)} + \frac{1}{C_m} \qquad (3\text{-}4)$$

where C_s ($\mu mole/m^2$) is the surface concentration of surfactant, a (mole/l) is a constant, C_m ($\mu mole/m^2$) is the surface concentration of surfactant at monolayer adsorption, and C is the surfactant concentration in mobile phase (mole/l). If the adsorption isotherm follows the Langmuir isotherm behavior, the plot of $1/C_s$ vs 1/C will be linear, and the value of the concentration of monolayer coverage can be estimated. But the linearized Langmuir adsorption isotherm plots of C14TAB, CTAB, and C18TAB, which are Figs. 3-18, 3-19, 3-20, and 3-21, did not exhibit a linear relationship between $1/C_s$ and 1/C. When making the plot at less concentrated regions for each isotherm (three points, at least), the intercepts, $1/C_m$, all were negative values even the coefficients of correlation were very close to 1 (0.9862 to 0.9998). This is, because after micelle has formed, increasing the surfactant concentration, the free surfactant concentration stays fairly constant. Therefore, there is not enough free surfactant to achieve monolayer coverage of the stationary phase. Also, the Langmuir isotherm is based on assuming equivalent adsorption sites, no solute-solute or solute-solvent interaction in either phase, both solute and solvent having equal molar surface area, and the adsorption film being monomolecular. In a LC column with micellar solution, these assumptions seem invalid. In the initial portion of these adsorption isotherms, which is below the CMC, the steepness of the rising portions of adsorption isotherms can be estimated by taking the ratio of surface coverage of surfactant to the CMC value. The steepness is C18TABs (with 10-cm column) ≈ C18TABs (with 15-cm column) > CTABs > C14TABs. For the hydrophobic stationary

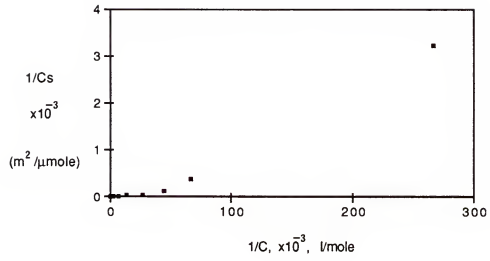


Fig. 3-18 Linearized Langmuir adsorption equation for C14TAB at 30°C

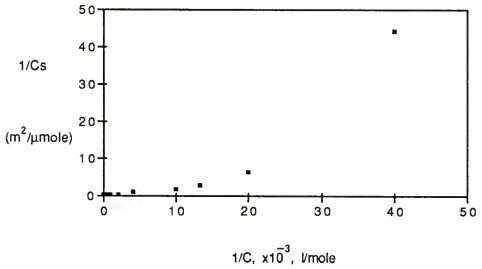


Fig. 3-19 Linearized Langmuir adsorption equation for CTAB at 30°C

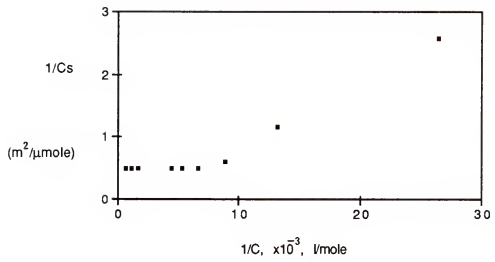


Fig. 3-20 Linearized Langmuir adsorption equation for C18TAB at 30°C

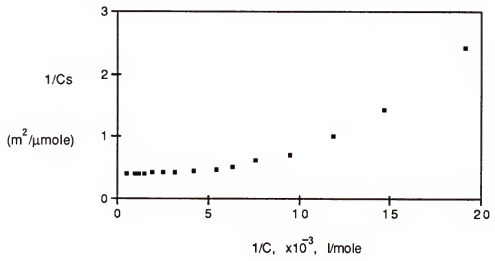
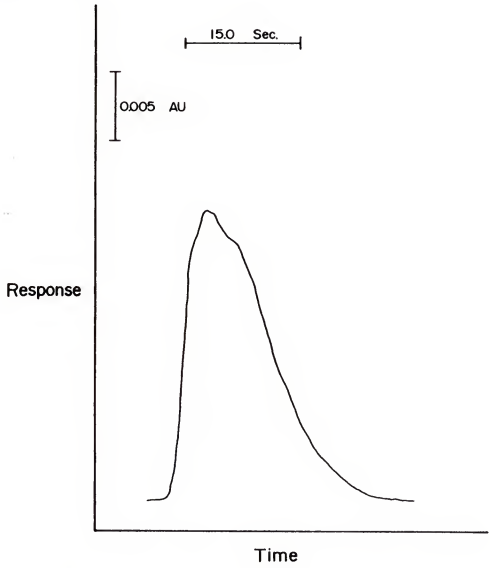
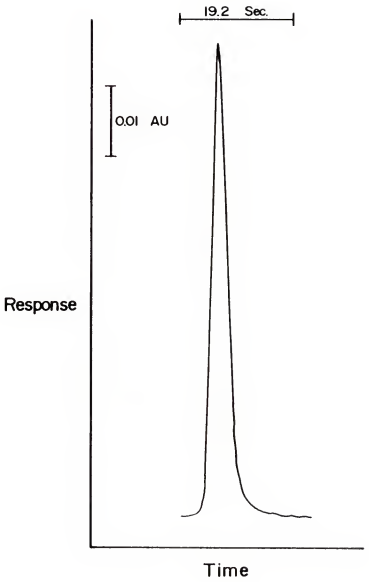


Fig. 3-21 Linearized Langmuir adsorption equation for C18TAB at 40°C

phase, the longer the methylene chain of the surfactant, the more favorable is the interaction. Therefore, C18TAB has the greatest steepness of all (34). For C18TAB with two different octyl columns, the equilibrium constants of different solutes seem to agree with each other well, but the fraction of surface coverage appears to be quite different, 69% for 15-cm column and 96% for 10-cm column, and also the CMC values from the two different columns are different by 23% with the 10-cm one yielding a higher CMC value. Since C18TAB is a cationic surfactant, the adsorption onto a surface can take place through either hydrophobic or ionic interaction or both. Ideally, the octyl column should provide only hydrophobic sites, but even after the octyl ligand is bonded onto the base silica particles, unless they are sufficiently end capped, they will still have residual silanol groups to take part in the adsorption process of the cationic groups onto the silanol groups, thereby increasing the overall surface coverage. This behavior can be justified by Fig. 3-22. Before the C18TAB solution was pumped through a new 10-cm column of the same type as used in the adsorption isotherm, the aniline peak appeared to be broad and exhibited a hump, indicating a mixed interaction mechanism was involved. After doing several experiments with the C18TAB micellar mobile phase in the same 10-cm column, aniline was injected again under the same conditions as previously, and the resultant peak shape appeared to be more symmetric. Apparently, the residual silanol groups had been suppressed. For the new 15-cm column, the aniline peak under the same conditions had a shape comparable to the more symmetric one for the 10-cm column.

Fig. 3-22 Chromatograms for aniline in 60/40 (methanol/H_2O), C-8 cartridge column: left, brand new column; right, after used in C18TAB micellar mobile phase


15.0 Sec.
0.005 AU
Response
Time


19.2 Sec.
0.01 AU
Response
Time

Equilibrium Constants

In micellar liquid chromatography, there is an additional factor, the micelle aggregate, one can manipulate during the separation. Therefore, besides its interaction between the mobile phase and stationary phase, the solute can also interact between the micelle and the bulk mobile phase, as well as between the micelle and the stationary phase (Fig. 3-23). In these experiments, C14TAB, CTAB, and C18TAB solutions in 3/97 1-propanol/water at concentrations above the CMC were used as mobile phases for three 15-cm and one 10-cm octyl columns.

There are two theoretical models which can be used to estimate either the solute equilibrium constant or solute partition coefficient from the measured chromatographic parameters. The first model is the equilibrium model (35), which can be expressed by the following equation:

$$1/k' = \frac{[M_m]K_2}{\phi[L_s]K_1} + \frac{1}{\phi[L_s]K_1} \qquad (3\text{-}5)$$

where k' is the capacity factor of the solute, $[M_m]$ is the micelle concentration (mole/l) of the surfactant ($[M_m]$ = [surfactant] - CMC), ϕ is the volume phase ratio of the stationary and mobile phases, $[L_s]$ is concentration of the stationary phase ligands, K_1 is the equilibrium constant of solute between the bulk mobile phase and the stationary phase, and K_2 is the equilibrium constant of the solute between the bulk mobile phase and a single surfactant molecule. The equilibrium constant of the solute between the bulk solution and the micelles then can be calculated by multiplying K_2 by the aggregation number of the surfactant.

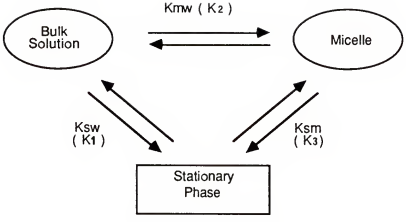


Fig. 3-23 Three-phase model for analytes at exchanging among micellar liquid chromatography

The second model is the the partition model (36), which can be expressed by the following equation:

$$\frac{V_s}{V_e - V_v} = \frac{v(K_{mw} - 1)}{K_{sw}} C_m + \frac{1}{K_{sw}} \qquad (3\text{-}6)$$

where V_s is the volume of stationary phase, V_e is the retention volume of the solute, V_v is the void volume of the column, v is the partial specific volume (ml/g) of the micelle in the solution, C_m is the micelle concentration (g/ml), K_{mw} is the partition coefficient of the solute between a single surfactant molecule and the bulk solution, and K_{sw} is the partition coefficient of the solute between the stationary phase and the bulk solution.

In equations 3-5 and 3-6, the difference between 1/k' and $V_s/(V_e - V_m)$ is only the factor, V_s/V_m, which is the volume phase ratio of the stationary and mobile phases. The equations are mathematically interrelated. Therefore, from the equilibrium constant, the partition coefficients can be obtained and vice versa. It is more straightforward to estimate the equilibrium constant, since only the solute capacity factor and micelle concentration are needed, whereas in the partition model, in addition to these factors, the partial specific volume of the micelle is also needed. In the equilibrium model, K_2 can be obtained by plotting 1/k' vs $[M_m]$, which has linear relationship, and then taking the ratio of the slope over the intercept. More hydrophobic compounds, such as benzene and anthracene, have smaller slopes and intercepts (Tables 3-8, 3-9, 3-10, and 3-11) than less hydrophobic ones. Smaller intercepts mean that there are stronger interactions of the solute with stationary phase. The more hydrophobic analyte, which also has smaller

Table 3-8 Slope and intercept of both models for analytes in C14TAB mobile phase at 30°C

	Equilibrium Model		Partition Model	
	Slope	Intercept	Slope	Intercept
Aniline	1.57	0.131	0.785	0.220
Acetophenone	1.33	0.104	0.664	0.0175
Phenol	1.30	0.0468	0.649	0.00786
Nitrobenzene	1.17	0.0496	0.583	0.00833
Benzene	1.12	0.0415	0.559	0.00696
Anthracene	0.867	0.0107	0.433	0.00180

Table 3-9 Slope and intercept of both models for analytes in CTAB mobile phase at 30°C

	Equilibrium Model		Partition Model	
	Slope	Intercept	Slope	Intercept
Aniline	1.48	0.104	0.680	0.0175
Acetophenone	1.25	0.0550	0.575	0.00924
Phenol	1.22	0.0272	0.564	0.00456
Nitrobenzene	1.14	0.0306	0.525	0.00515
Benzene	1.00	0.0234	0.461	0.00394
Anthracene	0.765	-0.00426	0.353	-0.000714

Table 3-10 Slope and intercept of both models for analytes in C18TAB mobile phase at 30°C

	Equilibrium Model		Partition Model	
	Slope	Intercept	Slope	Intercept
Acetophenone	1.95	0.0604	0.805	0.00979
Phenol	1.57	0.0334	0.648	0.00541
Nitrobenzene	1.45	0.0366	0.597	0.00592
Benzene	1.15	0.0246	0.473	0.00399

Table 3-11 Slope and intercept of both models for analytes in C18TAB mobile phase at 40°C

	Equilibrium Model		Partition Model	
	Slope	Intercept	Slope	Intercept
Acetophenone	1.78	0.539	1.09	0.0130
Phenol	1.19	0.418	0.728	0.0101
Nitrobenzene	1.35	0.0336	0.829	0.00811
Benzene	1.09	0.0254	0.669	0.00613

intercept, has the larger decrease in capacity factor with increasing micelle concentration. Of these three different cationic surfactants, the one with the longest chain length has a greater ability for reducing the retention of more hydrophobic compounds, like benzene than for the less hydrophobic compounds, like acetophenone. This shows that increasing the chain length of the surfactant will make hydrophobic solutes elute from the column faster than less hydrophobic ones. Dissolution of surfactant molecules in aqueous solution is dependent on the balance between the hydrophobic effect of the nonpolar hydrocarbon chain and the hydrophilic effect of the polar head group. Once the hydrocarbon chain length of the surfactant increases, its hydrophobic exclusion by the surrounding water also increases; therefore the surfactant molecules, in order to minimize exposure of the hydrocarbon chain to an aqueous environment, will start to aggregate and form micelles earlier. Therefore, for the same type of surfactants, longer chain length surfactants will have a lower CMC. Each ionic surfactant has a characteristic temperature called the Krafft point, which is the temperature where the water solubility of the surfactant is equal to the CMC. The Krafft point increases with surfactant chain length because the increase of the hydrocarbon chain length, which increases hydrophobicity as well, causes the reduction in the surfactant solubility. The higher Krafft point of C18TAB (Table 3-12) limits it from increasing the micelle concentration in order to obtain a reasonable retention for separations at the desired temperature. The K_2 values obtained are always larger for anthracene and benzene than for polar compounds like phenol and acetophenone. Therefore, they are also better able to interact with micelles more strongly than polar neutral

Table 3-12 Physical parameters of three cationic surfactants

Surfactant	Partial[a] Specific Volume (ml/g)	Aggregation[b] Number	Krafft Point, °C	CMC[c], M
C14TAB	1.002	75	--	3.5×10^{-3}
CTAB	0.9979	61	23[d]	9.2×10^{-4}
C18TAB	1.062	50[a]	30[a]	3.0×10^{-4}

[a] Estimated values.

[b] From reference 6.

[c] From reference 32.

[d] From reference 5.

compounds. For anthracene, since it interacts with the stationary phase so strongly, which means a small intercept value, the uncertainty will also increase for determination of the intercept. Like in CTAB, the intercept is negative. For a solute in same micellar mobile phase, no matter how different the stationary phase may be, ideally the K_2 values will be the same. From the K_{eq} values obtained in C18TAB micellar solution with two C8 columns from different manufacturers, it seems the prediction is followed with the exception of phenol. This might be due to the hydroxyl group on phenol molecule. Even the pKa of phenol is small, and the temperature difference might cause the slight difference in ioniza tion which makes noticeable change in the K_{eq} value with C18TAB micelle.

In partition model, the partial specific volume (v) is needed, and it can be estimated by the following equation:

$$v = \frac{V_t - [(W_t - W_m)/D]}{W_m} \qquad (3\text{-}7)$$

where V_t is the total volume (ml) of the micellar solution, W_t is the weight (g) of the micellar solution with volume of V_t, W_m is the weight of the micelle aggregates in the micellar solution with volume of V_t, and D is the density of the solvent (3/97, 1-propanol/water). Values are shown in Table 3-12.

In the partition model, the partition coefficient of solutes between stationary phase and bulk solution can be estimated by taking the reciprocal of the intercept. If the stationary phase had not been modified by the surfactant molecules, the K_{sw} values of the same solute would be expected to be the same in different micellar mobile phases. From Tables 3-13, 3-14, 3-15, and 3-16, certainly solutes

Table 3-13 Calculated K_{eq} and K_{sw} values for analytes in C14TAB micellar mobile phase

	K_{eq}*	K_{sw}**
Aniline	9.0×10^2	45
Acetophenone	9.6×10^2	57
Phenol	2.1×10^3	130
Nitrobenzene	1.8×10^3	120
Benzene	2.0×10^3	140
Anthracene	6.1×10^3	560

Note: Tetradecyltrimethylammonium bromide (C14TAB), M.W. 336.46 g/mole, CMC: 1.37×10^{-2} M, 30°C, v: 1.00 ml/g, aggregration number: 75, 15 cm, 5 μm, C8 column.

* Equilibrium constant between bulk solution and micelle.

** Partition coefficient between stationary phase and bulk solution.

Table 3-14 Calculated K_{eq} and K_{sw} values for analytes in CTAB micellar mobile phase

	K_{eq}*	K_{sw}**
Aniline	8.6×10^2	57
Acetophenone	1.4×10^3	110
Phenol	2.7×10^3	220
Nitrobenzene	2.3×10^3	190
Benzene	2.6×10^3	250

Note: Hexadecyltrimethylammonium bromide (CTAB), M.W. 364.46 g/mole, CMC: 6.73×10^{-4} M, 30°C, v: 0.98 ml/g, aggregration number: 61, 15 cm, 5 µm, C8 column.

* Equilibrium constant between bulk solution and micelle.

** Partition coefficient between stationary phase and bulk solution.

Table 3-15 Calculated K_{eq} and K_{sw} values for analytes in C18TAB micellar mobile phase (30°C)

	K_{eq}*	K_{sw}**
Acetophenone	1.6×10^3	100
Phenol	2.3×10^3	180
Nitrobenzene	2.0×10^3	170
Benzene	2.3×10^3	250

Note: Octadecyltrimethylammonium bromide (C18TAB), M.W. 392.52 g/mole, CMC: 1.49×10^{-4} M, 30°C, v: 1.06 ml/g, aggregration number: 50, 15 cm, 5 μm, C8 column.

* Equilibrium constant between bulk solution and micelle.

** Partition coefficient between stationary phase and bulk solution.

Table 3-16 Calculated K_{eq} and K_{sw} values for analytes in C18TAB micellar mobile phase (40°C)

	K_{eq}*	K_{sw}**
Acetophenone	$1.6x10^3$	77
Phenol	$1.4x10^3$	99
Nitrobenzene	$2.0x10^3$	120
Benzene	$2.1x10^3$	160

Note: Octadedcyltrimethylammonium bromide (C18TAB), M.W. 392.52 g/mole, CMC: $1.83x10^{-4}$ M, 40°C, v: 1.06 ml/g, aggregation number: 50, 10 cm, 5 μm C8 column.

* Equilibrium constant between bulk solution and micelle.

** Partition coefficient between stationary phase and bulk solution.

used in 10-cm column have different K_{sw} values from in 15-cm ones. But even in 15-cm columns, K_{sw} values in C14TAB are different from those in CTAB and C18TAB. This is because of the different percent coverage by different surfactants. While in CTAB and C18TAB, K_{sw} values are very close for each solute. This outcome mostly is attributed to the surface coverage by the surfactant molecules. In C14TAB, the stationary phase surface coverage is 66%, while for CTAB and C18TAB it is 89% and 69%, respectively. Therefore, the stationary phase coverage by C14TAB is not as great as for CTAB. For C18TAB, the surface coverage is less than for CTAB, but since C18TAB is two methylene units longer than CTAB, C18TAB can cover the stationary phase more effectively than CTAB can, which may be the cause of the small difference in the solute K_{sw} values for CTAB and C18TAB.

For aniline, the values obtained in the C18TAB experiments could not be fitted into equations 3-5 or 3-6. This deviation from the predicted trend is expected, since the pKa of the anilinium ion in pure aqueous solution is 4.6, the pH values of the mobile phases are between 4 to 6 (Tables 3-17, 3-18, and 3-19). Since these models are assumed to deal with neutral compounds, the effects of pH and electrostatic interactions are not taken into account in either model. Therefore, this deviation is expected for acidic and basic compounds. Yet this deviation did not appear in either C14TAB and CTAB. In order for such a deviation to take place, the electrostatic interactions must surpass the hydrophobic interaction (37). In other words, aniline must be present with certain ratio of [anilinium ion]/[aniline]. This ratio can be looked at in the same way as for

Table 3-17 pH Values for C14TAB in 3/97 (V/V, 1-propanol/water) at room temperature

[C14TAB], (mole/l)	pH
3.75×10^{-6}	5.20
1.50×10^{-5}	5.20
2.25×10^{-5}	5.20
3.75×10^{-5}	5.27
7.50×10^{-5}	5.40
1.50×10^{-4}	5.47
3.75×10^{-4}	5.43
1.79×10^{-3}	4.75
1.50×10^{-2}	4.49
3.00×10^{-2}	4.49
5.90×10^{-2}	4.25
7.50×10^{-2}	4.39
1.00×10^{-1}	4.49
1.52×10^{-1}	4.25

Table 3-18 pH Values for CTAB in 3/97 (V/V, 1-propanol/water) at room temperature

[CTAB], (mole/l)	pH
2.50×10^{-5}	4.68
5.00×10^{-5}	5.15
7.50×10^{-5}	5.00
1.00×10^{-4}	4.73
2.50×10^{-4}	4.86
5.00×10^{-4}	5.65
1.00×10^{-3}	5.50
2.50×10^{-3}	5.39
5.00×10^{-3}	5.15
7.50×10^{-3}	5.65
1.00×10^{-2}	5.50
2.00×10^{-2}	5.42
4.00×10^{-2}	5.52

Table 3-19 pH Values for C18TAB in 3/97 (V/V, 1-propanol/water) at room temperature

[C18TAB], (mole/l)	pH
3.78×10^{-5}	5.60
7.56×10^{-5}	5.87
1.13×10^{-4}	5.84
1.51×10^{-4}	5.94
1.89×10^{-4}	5.82
2.27×10^{-4}	5.94
6.05×10^{-4}	5.94
9.07×10^{-4}	5.84
1.51×10^{-3}	5.74

acid dissociation constant, pKa. In micellar solutions, the constants will shift because of the local interfacial polarity and the micellar surface potential change compared to the bulk solution (38, 39), which can be related to the equation below:

$$pKa_a = pKi_{(s)} - Y/59.16 \qquad \text{(at 25°C)} \qquad (3\text{-}8)$$

where pKa_a is the apparent acid dissociation constant, $pKi_{(s)}$ is the intrinsic acid dissociation constant at micelle surface, and Y is the surface potential of micelle in mv. For alkyltrimethyl-ammonium bromide surfactants, the longer the alkyl chain length, the higher the $pKi_{(s)}$ (38). From the deviation of C18TAB, it can be surmised that the pKa of anilinium ion was shifted to a higher value to a greater extent than in C14TAB and CTAB. Therefore in C18TAB, the ionic form of aniline dominated and caused the deviation.

Solvent Strength

The interaction between solute and solvent molecules can be the result of four types of interactions: dispersion, dipole, hydrogen bonding, and dielectric interactions. The resultant interaction of these four factors is termed as the polarity of the compound (39). For reversed phase liquid chromatography, the solvent strength decreases with increasing polarity.

For micellar mobile phases, the solvent strengths of SDS and CTAB in some concentrations have been estimated with respect to 100% and 90/10 (methanol/water) mobile phases. It is found that the micellar mobile phases have a weaker solvent strength (10). It is interesting to find out how the surfactant chain length affects the solvent

strength and also to broaden the comparison of solvent strength to broader scope of micellar solutions.

The equation used to estimate solvent strength (ε°) was derived by Snyder for normal phase adsorption chromatography (33). Later, Colin et al. adopted an equation to study the temperature effects on the solvent strength in reversed phase chromatography with pyrocarbon-containing stationary phase. The equation is

$$\log(\frac{k'_{s1}}{k'_{s2}}) = a(\varepsilon^{o}_{s2} - \varepsilon^{o}_{s1}) \qquad (3\text{-}9)$$

where k'_{s1} is the capacity factor for the solute in mobile phase s1; k'_{s2} is the capacity factor for the same solute in the mobile phase s2; a is the molecular area of the solute; and ε^{o}_{s1} and ε^{o}_{s2} are the solvent strength of mobile phases s1 and s2, respectively. If a particular mobile phase is chosen as a reference and its ε° value set at 0, then the sequence of the solvent strength for different mobile phases can be obtained.

In this experiment, 60/40 (methanol/water) was used as the reference mobile phase, and three different micellar mobile phase were used for comparison. The results are listed in Table 3-20. The ε° values obtained are negative values; therefore, the micellar solutions used are weaker mobile phases than the 60/40 (methanol/water) mobile phase. When ε° values were plotted versus the surfactant concentration, linear fitting of the data could be done with a coefficient of correlation of no less than 0.9874 (Table 3-21). From

Table 3-20 Calculated solvent strength for C14TAB, CTAB, and C18TAB micellar mobile phases

[C14TAB], M	Compound	HSA* ($Å^2$)	$\varepsilon°$ ($\times 10^{-2}$)
	Temperature: 30°C		
1.50×10^{-2}	Nitrobenzene	86	-1.27
	Benzene	110	-0.953
3.00×10^{-2}	Nitrobenzene	86	-1.15
	Benzene	110	-0.860
5.90×10^{-2}	Nitrobenzene	86	-0.942
	Benzene	110	-0.692
1.00×10^{-1}	Nitrobenzene	86	-0.738
	Benzene	110	-0.522
[CTAB], M			
4.00×10^{-2}	Nitrobenzene	86	-1.05
	Benzene	110	-0.769
8.00×10^{-2}	Nitrobenzene	86	-0.816
	Benzene	110	-0.583
1.00×10^{-1}	Nitrobenzene	86	-0.723
	Benzene	110	-0.502
1.50×10^{-1}	Nitrobenzene	86	-0.557
	Benzene	110	-0.369
[C18TAB], M			
3.02×10^{-3}	Nitrobenzene	86	-1.40
	Benzene	110	-1.06
9.07×10^{-3}	Nitrobenzene	86	-1.31
	Benzene	110	-0.978
1.81×10^{-2}	Nitrobenzene	86	-1.20
	Benzene	110	-0.873
3.02×10^{-2}	Nitrobenzene	86	-1.06
	Benzene	110	-0.768

Table 3-20--continued.

[C18TAB], M	Compound	HSA* ($Å^2$)	ε° ($\times 10^{-2}$)
	Temperature: 40°C		
1.06×10^{-3}	Nitrobenzene	86	-1.45
	Benzene	110	-1.13
2.00×10^{-3}	Nitrobenzene	86	-1.43
	Benzene	110	-1.12
4.01×10^{-3}	Nitrobenzene	86	-1.39
	Benzene	110	-1.09
9.99×10^{-3}	Nitrobenzene	86	-1.30
	Benzene	110	-1.01

Solvent strength (ε°) of micellar mobile phases in 3/97, 1-propanol/water.

Reference mobile phase: 60/40, methanol/water.

* Hydrocarbonaceous surface area.

Table 3-21 Slope and intercept of ε° vs surfactant concentration plot

	C14TAB[a]	CTAB[a]	C18TAB[a]	C18TAB[b]
Nitrobenzene				
Intercept	-0.0134	-0.0120	-0.0143	-0.0146
Slope	0.0623	0.0444	0.1238	0.1654
Coefficient of correlation	0.9942	0.9874	0.9987	0.9974
Benzene				
Intercept	-0.0101	-0.0089	-0.0108	-0.0115
Slope	0.0506	0.0361	0.1068	0.1356
Coefficient of correlation	0.9955	0.9879	0.9951	0.9997
Nitrobenzene				
Solvent strength[c]	-0.0072	-0.0076	-0.0019	0.0019
Concentration[d]	0.215	0.270	0.116	0.088
Benzene				
Solvent strength[c]	-0.0050	-0.0053	-0.0001	0.0021
Concentration[d]	0.200	0.247	0.101	0.085

[a] 30°C

[b] 40°C

[c] Assuming concentration being equal to 0.1 M, then fitted into the linearized equation to find ε°.

[d] Assuming the ε° being equal to 0, then from the linearized equation to find the concentration, unit in M.

Table 3-21, the C18TAB micellar solution has the largest slope among these three surfactants, meaning it has the greatest power for increasing the solvent strength per unit of concentration increasing. Since the solvent strength vs surfactant concentration plots have good linear correlation, the solvent strength at a particular surfactant concentration can be obtained and compared for these different chain length surfactants. In Table 3-21, at concentrations equal to 0.1 M, the C18TAB has the greatest solvent strength. Between the two C18TAB solutions at different temperatures, the higher temperature one has a greater solvent strength. In comparing the relative solvent strength for benzene and nitrobenzene, any conclusion might be misleading since the molecular surface area is not a definite value for either compound. From the equilibrium experiments and the solvent strength determinations, C18TAB solution seems to be the micellar solution of choice between these three surfactants for performing reversed phase separations. C18TAB solution has a tendency to decrease the retention of the more hydrophobic compounds to a greater extent than that for the less hydrophobic ones, which can speed up the separation and increase the sensitivity. Also, per unit of concentration increase for the C18TAB surfactant, the solvent strength increases more than for the other two shorter chain length micellar solutions, which can also benefit longer separations. But judging from experience in the handling of the C18TAB surfactant, its Krafft point is in the upper 20s°C, which presents a precipitation problem when working in an air conditioned environment without heating the mobile phases.

Optimization in Phenylthiohydantoin Amino Acids Separations

Phenylthiohydantoin amino acids (PTH-amino acids) are the products of Edman degradation of peptides and proteins. Separation of PTH-amino acids has been performed by several different separation techniques; among them, HPLC is the method of choice and reversed phase mode is the most frequently used (40).

Two elution systems, isocratic and gradient, have been used for PTH-amino acid separations. The isocratic elution systems have the advantage of simplicity, but often have long separation times (41). In gradient elution systems, the separation time can be reduced and good sensitivity can be obtained (42), but it is more complicated to obtain optimized conditions, and the theoretical linear solvent strength model is not always appropriate for every pair of PTH-amino acids to be separated (43). An optimization method has been proposed (44) which involves data for 190 peak pairs data and several days of computer cpu time for global optimization of the separation of the twenty PTH-amino acids. It seems that optimization of the PTH-amino acids separation is still not widely accepted. Recently, Foley and May (45) proposed a model for optimization of secondary chemical equilibria (SCE) in liquid chromatography which gives more reliable theoretical predictions of the optimized equilibrant concentrations. In micellar chromatographic separations, not only are the stationary phase and aqueous phase involved, but also the micelle aggregates take a vital role in the separation. This can be viewed as a secondary chemical equilibrium process; therefore this optimization scheme should be applicable to PTH-amino acids separation by micellar liquid chromatography.

The optimization is based on optimizing the resolution, which can be expressed as the product of an efficiency factor, a selectivity factor, and a retention factor. Those factors are roughly independent of one another. Among them, the selectivity factor is the most powerful and difficult one to optimize, thus this optimization procedure is intended to optimize selectivity.

For SCE, the relationship between analyte and solute can be expressed as follows:

$$AM \underset{\leftarrow}{\overset{\rightarrow}{}} A + M \tag{3-10}$$

and

$$K_e = \frac{[A][M]}{[AM]} \tag{3-11}$$

also

$$k' = F_{AM}k'_{AM} + F_A k'_A \tag{3-12}$$

where K_e is the equilibrium constant, [A] is the concentration of analyte, [M] is the micelle concentration, [AM] is the concentration of the micelle which is incorporated with the analyte, and k' is the observed capacity factor, which can be expressed as the weighted average of the analyte's limiting capacity factors (k'_{AM},k'_A) which are the capacity factors for analyte completely associated with micelle and analyte without associating with any micelle. F_{AM} and F_A are the stoichiometric fractions of the analyte in each of its forms. Therefore, k' can be expressed in terms of [M] as in equation

3-13, and k'_{AM}, k'_{A} can be obtained by plotting $k'(1 + K_e/[M])$ vs $(K_e/[M])$ according to equation 3-14,

$$k' = \{[M]/([M] + K_e)\}k'_{AM} + \{K_e/([M] + K_e)\}k'_{A} \qquad (3\text{-}13)$$

$$k'(1 + K_e/[M]) = k'_{AM} + (K_e/[M])k'_{A} \qquad (3\text{-}14)$$

The selectivity factor (α) is the ratio of two adjacent peaks' capacity factors, such that it is greater or equal to 1. Taking the ratio of k' (equation 3-13) for each analyte, the maximum (optimized) α can be estimated by taking the first derivative of capacity factor, and obtaining the optimized micelle concentration, $[M]_{opt}$. Assuming $k'_{AM(2)} = k'_{AM(1)}$ and $k'_{A(2)} = k'_{A(1)}$, then

$$[M]_{opt} = (K_{e(1)}K_{e(2)}k'_{A}/k'_{AM})^{1/2} \qquad (3\text{-}15)$$

where 1 and 2 refer to analyte 1 and analyte 2, respectively. This optimization scheme has been tested and verified in acid-base systems (45, 46).

In micellar liquid chromatography, the equilibrium constants of solutes can be estimated by using the equilibrium model described previously. With the constant coverage of the stationary phase by the surfactant above the CMC, the separation of PTH-amino acids might be optimized and also speeded up by several step gradient elutions.

In this experiment, SDS, CTAB, and Brij-35 were prepared in 3/97 (1-propanol/water) containing 0.01 M citric acid buffer with pH = 3. Three C-18 10-cm cartridge columns were used, one for each

surfactant. Columns were thermostatted by water with a temperature of 40°C.

From Table 3-22, 3-23, and 3-24, it can be seen that increasing the micelle concentration decreases the solute retention, and for ionized PTH-amino acids, responses to SDS and CTAB are quite different. For instance, when PTH-arginine hydrochloride is in SDS solution, the capacity factor can as high as 71.96, yet when it is in a CTAB solution, the capacity factor drops to less than 1; when it is in Brij-35, the capacity factor falls between the two, while for PTH-aspartic acid, the trend is just the opposite. It is obvious that ionic interactions are also taking effect regardless of the difference in solvent strength. For PTH-arginine hydrochloride in CTAB solution, the capacity factor increases slightly with increasing CTAB micelle concentration. This can be attributed mostly to uncertainty in the estimation of the void volume and some extent to the residual silanol groups in the stationary phase which might interact with the cationic character analyte. When plotting the capacity factor vs surfactant concentration (Figs. 3-24, 3-25, and 3-26), it is obvious that the total separation of the twenty PTH-amino acids is not obtained for each surfactant concentration examined. Therefore, some optimization scheme is needed. In this optimization procedure, the least resolved pair, PTH-leucine and PTH-isoleucine, were chosen for optimization. First of all, one must find the equilibrium constants for both analytes under micellar solution, and then find the limiting capacity factor of each analyte in both forms. Under SDS conditions, their limiting capacities and equilibrium constants were so close that the

Table 3-22 k' Values for twenty PTH-amino acids using SDS solutions as the mobile phase (at void volume of 0.62 ml)

	k'			
	0.025 M	0.050 M	0.10 M	0.20 M
PTH-Alanine	6.74	5.67	4.33	3.05
PTH-Arginine Hydrochloride	71.96	33.36	16.14	8.19
PTH-Asparagine	2.25	2.09	1.85	1.49
PTH-Aspartic Acid	2.54	2.32	1.96	1.65
PTH-S-Carboxymethylcysteine	4.66	3.94	3.05	2.25
PTH-Glutamic Acid	4.09	3.55	2.84	2.11
PTH-Glutamine	3.68	3.15	2.53	1.93
PTH-Glycine	4.27	3.81	3.15	2.37
PTH-Histidine	64.27	29.63	14.50	7.46
PTH-Isoleucine	28.89	17.57	10.38	6.27
PTH-Leucine	29.41	17.49	10.59	6.37
N-α-PTH-(ε-Phenylthiocarbamyl)-Lysine	24.12	13.70	7.75	4.53
PTH-Methionine	16.20	11.36	7.42	4.63
PTH-Phenylalanine	31.20	18.22	10.58	6.11
PTH-DL-Proline	22.41	15.93	10.51	6.92
PTH-Serine	2.61	2.45	2.11	1.68
PTH-Threonine	2.67	2.40	2.06	1.67
PTH-Tryptophan	21.80	13.41	7.81	4.51
PTH-Tyrosine	6.69	5.18	3.49	2.33
PTH-Valine	17.57	12.06	7.81	4.90

Table 3-23 k' Values for twenty PTH-amino acids using Brij-35 solutions as the mobile phase (at void volume of 0.55 ml)

	k'			
	0.025 M	0.050 M	0.075 M	0.10 M
PTH-Alanine	10.74	8.01	6.54	5.35
PTH-Arginine Hydrochloride	40.43	27.73	24.50	10.80
PTH-Asparagine	4.80	3.99	3.30	3.00
PTH-Aspartic Acid	9.24	6.74	5.70	4.60
PTH-S-Carboxymethylcysteine	13.83	9.02	6.74	5.84
PTH-Glutamic Acid	10.63	7.50	6.19	5.15
PTH-Glutamine	5.53	4.21	3.59	3.18
PTH-Glycine	7.73	6.14	5.20	4.67
PTH-Histidine	23.37	12.41	12.17	10.87
PTH-Isoleucine	34.16	19.37	14.05	11.22
PTH-Leucine	35.37	20.10	14.82	11.84
N-α-PTH-(ε-Phenylthiocarbamyl)-Lysine	40.39	21.24	14.78	11.75
PTH-Methionine	23.29	14.34	10.41	8.71
PTH-Phenylalanine	35.29	19.18	14.05	11.02
PTH-DL-Proline	17.86	11.81	9.00	7.62
PTH-Serine	5.83	4.50	3.81	3.57
PTH-Threonine	21.83	12.72	9.73	7.90
PTH-Tryptophan	37.35	19.37	13.79	11.06
PTH-Tyrosine	21.64	12.58	8.91	7.18
PTH-Valine	22.85	13.86	10.41	8.65

Table 3-24 k' Values for twenty PTH-amino acids using CTAB solutions as the mobile phase (at void volume of 0.62 ml)

	k'			
	0.025 M	0.050 M	0.10 M	0.15 M
PTH-Alanine	15.10	10.03	6.23	4.61
PTH-Arginine Hydrochloride	0.55	0.61	0.71	0.77
PTH-Asparagine	9.06	6.26	4.13	3.08
PTH-Aspartic Acid	23.06	12.23	6.58	4.52
PTH-S-Carboxymethylcysteine	27.00	13.45	6.94	4.69
PTH-Glutamic Acid	15.94	9.03	5.29	3.74
PTH-Glutamine	6.81	4.97	3.27	2.56
PTH-Glycine	10.13	7.39	4.97	3.76
PTH-Histidine	0.42	0.29	0.47	0.42
PTH-Isoleucine	41.77	22.52	12.37	8.37
PTH-Leucine	42.74	23.26	12.90	8.82
N-α-PTH-(ε-Phenylthiocarbamyl)-Lysine	63.26	32.23	16.82	11.26
PTH-Methionine	29.65	17.00	9.42	6.58
PTH-Phenylalanine	44.26	23.03	12.39	8.35
PTH-DL-Proline	25.29	15.42	9.13	6.45
PTH-Serine	8.74	6.35	4.24	3.26
PTH-Threonine	7.39	5.55	3.76	2.42
PTH-Tryptophan	57.87	29.29	15.45	10.42
PTH-Tyrosine	31.61	17.77	10.06	7.06
PTH-Valine	29.52	16.81	9.47	6.77

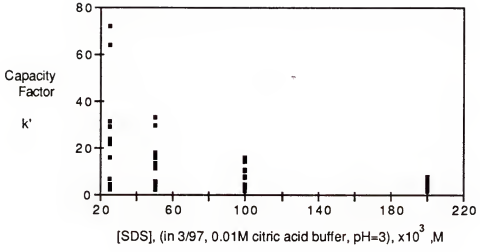


Fig. 3-24 k' vs [SDS] for twenty PTH-amino acids

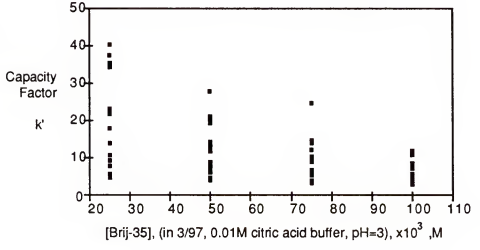


Fig. 3-25 k' vs [Brij-35] for twenty PTH-amino acids

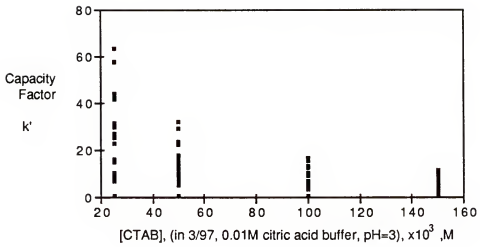


Fig. 3-26 k' vs [CTAB] for twenty PTH-amino acids

SDS micellar mobile phases were not able to separate them. Under CTAB conditions, the optimized experimental condition occurred at a concentration just less than that of the calculated optimized one. This may be due to the assumptions made in the calculation as well as the estimation error in the equilibrium constants for each analyte. In Brij-35, it seems that selectivity increases with decreasing concentration, but it is very possible that the optimized concentration was missed because there were only three data points. For the PTH-serine and PTH-asparagine pair in CTAB solutions (Table 3-25), the selectivity increases as the concentration decreases. This may be due to unsatisfactory linear curve fitting of PTH-serine data by equation 3-14, since the coefficient of correlation was 0.986. Since the PTH-serine carries a hydroxyl group, it may also interact with residual silanol groups in the stationary phase, giving a mixed retention mechanism instead of single interaction with surfactant modified stationary phase.

In the optimization of PTH-amino acids' separation, since some PTH-amino acids are carrying a charge, in order for this optimization scheme to be applicable, those charges need to be suppressed. Using micellar solutions as the mobile phases, if several difficult pairs of PTH-amino acids separations could have been optimized, because the stationary phase is constantly covered by the surfactant which eliminates the lengthy re-equilibration to initial conditions, the optimized micellar concentrations can be performed in a stepwise gradient elution. From the results obtained above, the outlook is encouraging, but more detailed examinations are still needed in order

Table 3-25 Optimization of twenty PTH-amino acids

Surfactant	Concentration, M	Capacity Factor, k'	Selectivity
	[For PTH-isoleucine (1) and PTH-leucine (2)]		
SDS	0.0126_{opt}	(1) 42.1 (2) 42.1	1
	0.0166	(1) 35.7 (2) 35.7	1
	Limiting Capacity Factor:	$k'_{A(1)} = 943.6$, $k'_{AM(1)} = 4.79$ $k'_{A(2)} = 940.5$, $k'_{AM(2)} = 4.79$	
	Equilibrium Constant:	$K_{e(1)} = 5.49 \times 10^{-4}$ $K_{e(2)} = 5.60 \times 10^{-4}$	
Brij-35	0.00417	(1) 90.3 (2) 93.8	1.039
	0.00835_{opt}	(1) 67.9 (2) 70.2	1.035
	0.0167	(1) 44.2 (2) 45.4	1.027
	Limiting Capacity Factor:	$k'_{A(1)} = 2310$, $k'_{AM(1)} = 3.77$ $k'_{A(2)} = 2130$, $k'_{AM(2)} = 4.25$	
	Equilibrium Constant:	$K_{e(1)} = 3.34 \times 10^{-4}$ $K_{e(2)} = 3.70 \times 10^{-4}$	
CTAB	0.00308	(1) 164.0 (2) 171.7	1.047
	0.00463	(1) 130.6 (2) 137.8	1.055
	0.00721	(1) 104.0 (2) 137.8	1.096
	0.00806_{opt}	(1) 97.7 (2) 102.4	1.047
	0.0154	(1) 60.1 (2) 62.6	1.042
	Limiting Capacity Factor:	$k'_{A(1)} = 7440$, $k'_{AM(1)} = 2.38$ $k'_{A(2)} = 6370$, $k'_{AM(2)} = 2.74$	
	Equilibrium Constant:	$K_{e(1)} = 1.31 \times 10^{-4}$ $K_{e(2)} = 1.55 \times 10^{-4}$	

Table 3-25--continued.

Surfactant	Concentration, M	Capacity Factor, k'	Selectivity
	[For PTH-serine (3) and PTH-asparagine (4)]		
CTAB	0.00108	(3) 12.7 (4) 15.0	1.175
	0.00463	(3) 12.6 (4) 13.5	1.077
	0.00802_{opt}	(3) 11.6 (4) 12.5	1.075
	0.0116	(3) 11.5 (4) 11.5	1
	0.0231	(3) 8.68 (4) 8.68	1
	Limiting Capacity Factor:	$k'_{A(3)} = 195$, $k'_{AM(1)} = 2.58$	
		$k'_{A(4)} = 253$, $k'_{AM(2)} = 2.28$	
	Equilibrium Constant:	$K_{e(3)} = 6.97 \times 10^{-4}$	
		$K_{e(4)} = 8.38 \times 10^{-4}$	

to fully utilize micellar mobile phases in the optimization of PTH-amino acids' separations.

CHAPTER IV
CONCLUSION AND FUTURE WORK

In reversed phase liquid chromatography, since there is no further change in adsorption of surfactant on the stationary phase, the amount of surfactant adsorbed onto the stationary phase is fairly constant above the CMC. Therefore, no re-equilibration is expected after each gradient run for those surfactants studied in the adsorption isotherms. For alkyltrimethyl ammonium bromide micellar solutions, the solvent strength increases with the increase in surfactant concentration and also longer alkyl chain length. The micellar solution under the same surfactant concentration has greater solvent strength. But due to the limitation of the solubility and the solution viscosity, it will be difficult just to increase the surfactant concentration in order to match the solvent strength of hydroorganic mobile phases with higher percentage of organic modifier. For zwitterionic surfactant, Z3-16, despite insensitive character, it only showed the anionic surfactant character. This might be due to the positive charge being embedded far inside the hydrophobic surroundings and preventing it from contacting the hydrophilic environment. Optimization of the selectivity of the PTH-amino acid pair through the secondary chemical equilibrium basis gave good estimation of the optimized surfactant concentration for CTAB, but not for Brij-35. A method for estimating the CMC has been

proposed (47). It utilizes the plot of selectivity vs surfactant concentration. When micellization occurs, the selectivity goes to the maximum. From the optimization studies, some of the experimental results show this type of tendency. Perhaps there are several optimized concentrations, CMC being one of them, of micellar solutions for a pair of analytes. In this study, the calculated optimized concentrations are very close to the CMC; if one selectivity is higher than the other, the lower one might be overshadowed by the other. But more studies will be needed to elucidate this connection. Micellar mobile phases are very suitable for several applications which are not for hydroorganic mobile phases. But in the solvent strength category, micellar mobile phases are weaker than hydroorganic ones. For micellar solutions of the same type of surfactant, the longer the carbon chain, the stronger the mobile phase. The longer carbon chain means increase in the hydrophobicity. For perfluorinated surfactant, it possesses higher hydrophobicity than the hydrocarbon counterpart. It might also have a greater solvent strength. But it is only a little study in the micelle region (48), further studies for the perfluorinated micelle will be needed. Zwitterionic surfactant has been used successfully in ion-pair chromatography to separate nucleotides (24, 25) and optical isomers (49). The zwitterionic surfactants used had charged groups in each end of the molecule; therefore, both head groups could access the bulk mobile phase with little steric problem. For the future study of the zwitterionic micellar solutions, the shorter carbon chain surfactant than Z3-16 will be more adequate.

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Associate Professor of Chemistry

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Vaneica Young
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April, 1988

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